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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 000711-0025	**FOR FURTHER ACTION** See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/CA 03/00499	International filing date *(day/month/year)* 04.04.2003	Priority date *(day/month/year)* 05.04.2002
International Patent Classification (IPC) or both national classification and IPC A61K9/51		
Applicant UNIVERSITE DE MONTREAL et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 03.11.2003	Date of completion of this report 05.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer von Eggelkraut-Gotta Telephone No. +31 70 340-4732

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1, 2, 4, 5, 7, 8, 11, 12, 14, 15, 19, 20, 22-36	as originally filed
3, 6, 9, 10, 13, 16, 17, 18, 21	filed with telefax on 03.11.2003

Claims, Numbers

1-9, 18-25, 39-44	as originally filed
10-17, 26-38	filed with telefax on 03.11.2003

Drawings, Sheets

1-25	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 21,37-40 with respect to industrial applicability

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 21,37-40 with respect to industrial applicability

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-3, 13-21, 37-43 (all partially) .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-12,22-36,41-44
	No:	Claims	1-3,13-21,37-40
Inventive step (IS)	Yes:	Claims	4-12,22-36,42-44
	No:	Claims	1-3,13-21,37-41
Industrial applicability (IA)	Yes:	Claims	1-20,22-36,41-44
	No:	Claims	

2. Citations and explanations

see separate sheet

III. Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1

1.1 Claims 21,37-40 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

V. Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

For the assessment of the present claims 21,37-40 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2 Reference is made to the following documents:

D1: US-A-6 139 870 (VERRECCHIA THIERRY) 31 October 2000 (2000-10-31)
D2: US-A-5 683 723 (BAZILE DIDIER ET AL) 4 November 1997 (1997-11-04)
D3: WO 01/12718 A (SEO MIN HYO ;CHOI IN JA (KR); SAMYANG CORP (KR)) 22 February 2001 (2001-02-22)
D4: NAKADA Y ET AL: "LONG-CIRCULATING NANOPARTICLES USING BIODEGRADABLE ABA TRIBLOCK COPOLYMERS CONTAINING POLY(L-LACTIC ACID) A-BLOCKS ATTACHED TO CENTRAL POLY(OXYETHYLENE) B-BLOCKS" PHARMACEUTICAL SCIENCES, LONDON, GB, vol. 3, no. 10, October 1997 (1997-10), pages 479-481, XP000783648 ISSN: 1356-6881
D5: RYU JAE-GON ET AL: "Clonazepam release from core-shell type nanoparticles of poly(epsilon-caprolactone)/poly(ethylene glycol)/poly(epsilon-caprola ctone) triblock copolymers" INTERNATIONAL JOURNAL OF PHARMACEUTICS (KIDLINGTON), vol. 200, no. 2, 10 May 2000 (2000-05-10), pages 231-242,

XP002257858 ISSN: 0378-5173

D6: PANOYAN AVEDIS, HILDGEN PATRICE: "Vecteurs polymériques injectables pour l'administration des anticancéreux" CONGRÈS DE L'ACFAS 2000 - COMMUNICATION PRÉSENTÉE AU CONGRES, [Online] page 1, XP002257860 Retrieved from the Internet: URL:http://www.acfas.ca/congres/congres68/ S1080.htm> [retrieved on 2003-10-14]

D7: US-A-6 007 845 (GREF RUXANDRA ET AL) 28 December 1999 (1999-12-28)

3 **NOVELTY** (Art. 33(2) PCT)

3.1 The document D1 discloses (see whole document) nanospheres < 100 nm comprising a PLA-PEG diblock copolymer and an anticancer agent. The subject-matter of claims 1-3, 13-17, 19-21, 37-39 is therefore not new.

3.2 The document D2 discloses (see whole document) stealthy biodegradable nanospheres < 500 nm comprising a PLA-PEG diblock copolymer. The subject-matter of claims 1-3, 13-21 and 37-39 is therefore not new.

3.3 The document D3 (see page 4, paragraph 4 - page 5, paragraph 1; page 5, last paragraph - page 6, paragraph 3; page 8, paragraph 2; page 10, paragraph 2, examples 10, and 11) discloses polymeric micelles of PEG-PLA-PEG or PEG-PLDO-PEG triblock copolymers and anticancer drugs. Polymeric micelles are considered to be nanospheres. The subject-matter of claims 1-3, 13-17, 20,21 and 37-39 is therefore not new.

3.4 The document D4 discloses (see whole document) nanospheres of 192 and 180 nm comprising PLA-POE-PLA triblock copolymer and progesterone. The subject-matter of claims 1-3, 13-21 and 37-39 is therefore not new.

3.5 D5 discloses (see page 232, right-hand column, paragraph 3 - page 233, left-hand column, paragraph 2; page 235, left-hand column, last paragraph - right-hand column, paragraph 1) nanospheres of polycaprolactone-PEG-polycaprolactone triblock copolymers and clonazepam, with a particle size of 32-60 nm. The subject-matter of claims 1-3, 13,15-17,20,21 and 37-39 is therefore not new.

3.6 D6 discloses (see whole document) nanospheres comprising PLA-PEG copolymer

nanosphere < 800 nm with zero zeta potential. The nanospheres are loaded with anticancer drug. The subject-matter of claims 1-3, 13-21, and 37-39 is therefore not new.

3.7 D7 discloses (see column 3, line 66 - column 4, line 22; column 6, line 63 - column 7, line 11; column 9, lines 19-39; column 12, line 65 - column 13, line 4; example 20; claims 6, 8, 10, 11, 13, 14, 20, 24, 26, 28, and 29) nanospheres of 200 nm, comprising non-linear multiblock copolymers of PEG-PLA and an antibody for targeting. The subject-matter of claims 1,3,17-21,37,39, and 40 is therefore not new.

3.8 The present application does not meet the requirements of Article 33(2) PCT because the subject-matter of claims 1-3,13-21,37-40 is not new.

3.9 In view of the prior cited, claims 41-43 appear to be novel and meet therefore the requirements of Art. 33(2) PCT.

4 **INVENTIVE STEP** (Art. 33(3) PCT)

4.1 The subject-matter of claims 1-3,13-21,37-40 is not new and therefore not inventive.

4.2 The document D2, which is considered to represent the most relevant state of the art, discloses (see example 2, second method of preparation) a method of preparation of biodegradable nanospheres comprising dissolving a PLA-PEG diblock copolymer in ethyl acetate and dispersing the organic phase in water in a homogenization chamber producing an emulsion. The ethyl acetate is evaporated. The subject-matter of claim 41 differs in that the organic phase does not comprise a pharmaceutical compound and that the nanospheres are not collected by centrifugation or dialysis.

4.3 The document D4, which can also be considered to represent the most relevant state of the art, discloses (see page 479, right-hand column, last paragraph - page 480, left-hand column, first paragraph) a method of preparation of biodegradable nanospheres comprising dissolving PLA-PEG-PLA triblock copolymer and progesterone in dichloromethane and injecting this solution into a polyvinyl alcohol solution followed by homogenization (a homogenization chamber is implicitly disclosed), producing an emulsion. The dichloromethane is evaporated and the nanospheres were separated by gel-permeation chromatography. The subject-matter of claim 41 differs in that the nanospheres are not collected by centrifugation or

dialysis.

4.4 These additional features of said claim are either directly known from documents D1-D5, or are a combination of features obvious to the man skilled in the art in consideration of the disclosure of the prior art named in the present proceedings, or they concern only minor modifications which lie within the normal practice of the man skilled in the art. The subject-matter of claim 41 therefore lacks an inventive step (Article 33(3) PCT).

4.5 It appears that the subject-matter of claims 42 and 43 is new and inventive (Articles 33(2) and 33(3) PCT).

5 **Certain defects**

5.1 The term "stealthy" used in claims 1-3, 13-17, 21, 37 and 41 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

5.2 The term "multiblock copolymer" used in claims 1-3, and 41 has no well-recognised meaning and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT). The expression is considered to encompass diblock and triblock copolymers per se, as well as polymers comprised of coupled triblock copolymers.

5.3 Claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The following functional statement does not enable the skilled person to determine which technical features are necessary to perform the stated function: "entangled with the multiblock copolymer to give rigidity to the nanospheres". This expression is not considered to be limiting the scope of the claim.

c) reacting the polyethylene glycol having terminal dichloride acid functions obtained in step b) with the PLA-PEG-PLA triblock polymer obtained in claim 34 by making use of polycondensation reaction so as to obtain a multiblock copolymer according to the invention.

A fifth object of the invention is to provide an improved method for preparing a PLA-PEG-PLA multiblock copolymer of formula (I):

$$ABA\text{-}(c\text{-}ABA)_n\text{-}c\text{-}ABA \qquad (I)$$

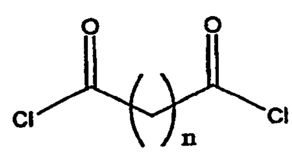
wherein

- n is a number equal or higher than 2;
- ABA is a PLA-PEG-PLA triblock; and
- c is a carboxylic diacid.

said method comprising the steps of:

a) preparing a PLA-PEG-PLA triblock;

b) mixing the PLA-PEG-PLA triblock prepared in step a) with a diacid of formula (II):



(II)

wherein n is a number equal to or greater than 1; and

c) subjecting the mixture of step b) to a polycondensation reaction with the presence of a dicyclohexylcarboxydiimide reagent and/or a chemical equivalent thereof, said catalyst improving the efficiency of the reaction, thereby allowing to obtain the requested multiblock copolymer.

A sixth object of the invention is to provide a method for delivering a pharmaceutical compound into a mammal, said method comprising the step of:

administering to the mammal a stealthy polymeric biodegradable nanosphere according to the invention loaded with a therapeutically effective amount of the pharmaceutical compound.

Figure 11 is the chemical formula of a multiblock polymer according to the invention.

Figure 12 A is a micrograph representing the nanosphere according to the invention after the release period of twenty-nine days.

Figure 12 B is a micrograph representing a nanosphere according to the invention that underwent degradation in a phosphate buffer at 37°C.

Figure 13 is a graph representing the weight loss of the bulk polymer used according to the invention.

Figure 14 is a graph representing the typical pore size distribution of nanospheres according to the invention.

Figure 15 is a bar graph representing the porosity (cm^3/g) of nanospheres made of various blends of PLA and multiblock polymers.

Figure 16 is a graph representing the proliferation of B16 cells in the presence of different components.

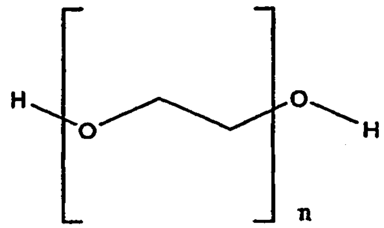
Figure 17 is graph representing the *in vitro* release of Rhodamine from nanospheres according to the invention in a phosphate buffer at 37°C.

Figure 18 is a graph representing the plasmatic concentration of Rhodamine after IV injection of nanosphere according to the present invention.

Figure 19 is a graph representing the concentration of Rhodamine in different organs.

Figure 20 are bar graphs representing the behavior of phagocytic cells in the presence of stealthy nanospheres according to the present invention.

Monomer B (PEG) can be obtained from the following compound:

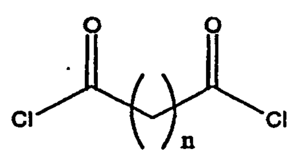


B

Wherein n represent a number between 200 and 2000.

Typically, the first step consists of mixing together one or several compounds of the A type with a compound of the B type. The compounds are polymerized by polycondensation under an inert atmosphere at a temperature of 160°C to 180°C for 2 to 6 hours. A tin-based catalyst such as tin octanoate or tetraphenyltin. The polymer ABA so obtained is dissolved in acetone and precipitated with water. The precipitate is then washed and dried.

The most common method for synthesizing a PLA-PEG-PLA multiblock copolymer from the ABA polymer is the method developed by Dupont in the seventies. Briefly, the ABA triblock polymer is placed in a round bottom flask in presence of a diacid chloride. Following a polymerization by polycondensation, and elimination of HCl, a multiblock ABA copolymer (ABA-c-ABA-c-ABA-c-ABA) is obtained. Suitable diacid chloride have the following formula (II):



(II)

Preferred diacid chlorides include: propanedioic acid, butanedioic acid, pentanedioic acid, etc.

Interestingly, the present inventors have found that the efficiency of the method is greatly improved when dicyclohexylcarboxydiimide (DCC) is used as a reagent in the reaction. Therefore, the present invention encompasses the use of DCC as well as chemical equivalents such as EDC (1-[3-dimethylaminopropyl]-3-

ethyl carbodiimide) for synthesizing a PLA-PEG-PLA multiblock copolymer from ABA polymers.

ii) Novel polyester-polyethylene multiblock copolymer and method for synthesizing the same

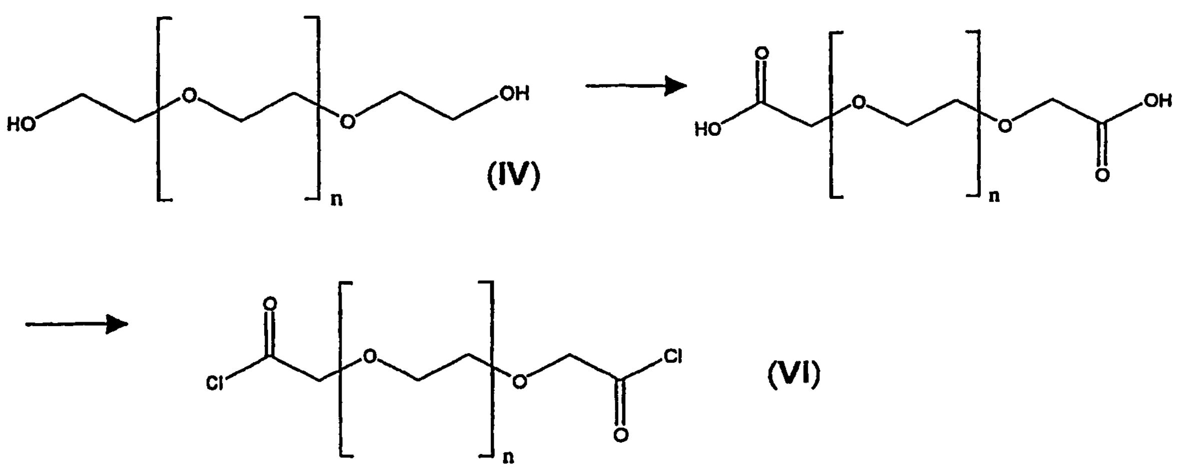
According to another aspect, the invention provides a multiblock copolymer that is composed of alternate blocks of polyester and of polyethylene glycol. According to the invention, these blocks are arranged according to the following manner:

$$\text{ABA-B'- ABA-B'-ABA-B'-ABA} \qquad \textbf{(III)}$$

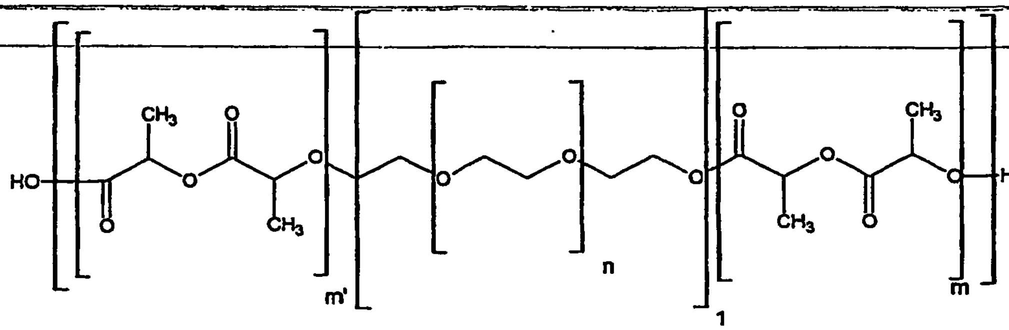
wherein A is a polyester, B is a polyethylene glycol and B' is a dicarboxylic polyethylene.

A non-exhaustive list of suitable polyesters includes polylactic acid (PLA); polylactic-co-glycolic acid (PLGA); polycaprolactone (PCL), and polyhydroxy butyrate. A non-exhaustive list of suitable polyethylenes includes polyethylene oxides (PEO) such as polyethylene glycol (PEG). A non-exhaustive list of suitable dicarboxylic polyethylene includes dichloride dicarboxylic PEG and dibromide dicarboxylic PEG. More preferably, the polyester consists of PLA, the polyethylene consists of PEG and the dicarboxylic polyethylene consists of dichlorine dicarboxylic PEG.

Preferably, the multiblock copolymer is synthesized by using PEG as the polyethylene. According to this embodiment, commercially available PEG is oxidized into a dicarboxylic PEG, then a dichloride acid is formed:



(IV) (V) (VI)



(VII)

According to this embodiment, these blocks are arranged according to the following manner:

ABA-c-ABA-c-ABA-c-ABA (I)

wherein "ABA" is the PLA-PEG-PLA triblock and "c" is a carboxylic diacid (e.g. butanedioic acid, propanedioic acid, pentanedioic acid (IUPAC nomenclature)).

According to another, more preferred embodiment, the multiblock copolymer is composed of alternate blocks of polyester and polyethylene glycol. According to this embodiment, these blocks are arranged according to the following manner:

ABA-B'- ABA-B'-ABA-B'-ABA (III)

wherein A is a polyester, B is a polyethylene glycol and B' is a dicarboxylic polyethylene.

A non-exhaustive list of suitable polyesters includes polylactic acid (PLA); polylactic-co-glycolic acid (PLGA); polycaprolactone (PCL), and polyhydroxy butyrate. A non-exhaustive list of suitable polyethylene includes polyethylene oxides (PEO) such as polyethylene glycol (PEG). A non-exhaustive list of suitable dicarboxylic polyethylene includes dichloride dicarboxylic PEG and dibromide dicarboxylic PEG. More preferably, the polyester consists of PLA, the polyethylene consists of PEG and the dicarboxylic polyethylene consist of dichloride dicarboxylic PEG.

ii) Polyester

Preferably, the nanospheres comprise about 0.1% to 99% of a polyester. The polyester, entangled with the multiblock copolymer, is useful for increasing the rigidity of the nanospheres. A non-exhaustive list of suitable polyester

described herein can be used in the practice for testing of the present invention, the preferred methods and materials are described.

Example 1: Synthesis and characterization of novel PLA-PEG multiblock copolymer

Introduction

Biodegradable polymers are studied in an increasing number of medical applications. They are used as drug carriers, controlled release systems, etc. Some authors are interested in the possibilities that a copolymer consisting of polylactic acid (PLA) and polyethylene glycol (PEG) can offer. A multiblock copolymer composed of PLA and PEG is of considerable interest as a drug carrier, since the PLA segments could provide rigidity, while the PEG portions confer stealth behavior (R.H. Muller. CRC Press Inc., Boca Raton, Florida, 1991: 45-46). PEG can offer a certain degree of hydrophilicity to the polymer that can be useful if we want to use it as a carrier for an hydrophilic drug. But the current ring-opening polycondensation of (D,L)-lactide in the presence of PEG can only produce an A-B-A triblock copolymer where the B block (PEG) is trapped between two A blocks (PLA).

We propose here an efficient synthesis method for a polyester-polyethylene multiblock copolymer where the polyester (A) blocks alternate with polyethylene (B) blocks to form a repetitive sequence.

Experimental methods

i) Materials

Polyethylene glycol (molecular weight 400), (D,L)-lactide, tetraphenyltin and adipic acid were purchased from Aldrich Chemical Company, Inc. (Oakville, Ont., Canada) and were dried under vacuum in the presence of phosphorus pentoxide for 24 hours prior to use. N,N-dimethylformamide was distilled over calcium hydride and kept on a 4Å molecular sieve prior to use. Thionyl chloride and pyridine were used as received from Aldrich Chemical Company.

ii) Preparation of triblock PLA-PEG-PLA copolymer

The triblock polymer was synthesized by a ring-opening polycondensation of (D,L)-lactide in the presence of PEG, as described by Cohn and Younes (*J. Biomed. Mater.Res.* **22**(11): 993-1009 (1988)). PEGs with different molecular weight were used. Briefly, 8.3 mmol of PEG (molecular weight 200, 400 or 1500) were added to 158.3 mmol of (D,L)-lactide (molecular weight 144,13) in a round bottom, single neck flask. Tetraphenyltin 0.01% was used as a catalyst. The reaction was carried at 180°C for 6 h under an argon-inert atmosphere. The resulting polymer was precipitated in water from acetone, removing any un-reacted PEG or (D,L)-lactide. The polymer was then dried under vacuum with phosphorus pentoxide.

iii) Preparation of a multiblock (PLA-PEG-PLA)$_n$ copolymer

The triblock copolymer (3 mmol) and adipic acid (3 mmol) were dissolved in N,N-dimethylformamide (40 ml) under an argon-inert atmosphere. A solution of thionyl chloride (15 mmol) in pyridine (15 ml) was added at 0°C over a period of 30 minutes. The temperature was brought to 20°C over a period of 10 hours, under magnetic stirring. The polymer was then precipitated in water and washed several times to remove any trace of solvent. Its structure is shown in Figure 1.

iv) Contact angle measurements

Contact angle measurements were made using a Tantec CAM-MICRO™ contact angle meter. For each copolymer, 200 mg was dissolved in 3 ml of dichloromethane, and a thin film was cast on a glass slide. The films were dried under vacuum to remove any trace of solvent. Polylactic acid was used as a reference for the contact angle measurement. Contact angle measurements were made at 0 and 420 seconds.

Results and discussion

^{1}H- NMR, using a Bruker 400 MHz spectrometer showed a typical spectrum for the triblock copolymer with peaks at 5.2 ppm corresponding to the tertiary PLA proton, at 3.6 ppm for the protons of the repeating units in the PEG chain, at 4.3

ppm for the PEG connecting unit to the PLA block, and at 1.5 ppm for the pendant methyl group of the PLA chain (not shown). For the multiblock copolymer showed in **Figure 6**, peaks corresponding to the protons in the adipic acid chain were detected at 3.0 and 2.3 ppm (see **Figure 7**).

Molecular weight **(Table 1)** was mesured by gel permeation chromatography using a Waters™ spectrometer. Molecular weight around 2000 Da for the triblock copolymer and 10 000 Da for the multiblock copolymer showed that the blocks were covalently bounded together.

Table 1: Molecular weight measurements

TRIBLOCK	Mn	Mw	I
PEG 200	1474.17	2151.45	1.46
PEG 400	900.84	1285.34	1.43
PEG 1450	2835.22	3595.42	1.27
MULTIBLOCK			
PEG 200	4357.02	9657.48	2.22
PEG 400	3646.71	8537.77	2.34
PEG 1450	6607.95	12040.6	1.82

Contact angle measurements **(Table 2)**, show that the polycondensation of PLA with PEG reduce the contact angle thus augmenting the hydrophilicity of the copolymer compared to PLA alone.

Table 2: Contact angle measurements

Polymer	Contact angle (t = 0s)	Contact angle (t = 420s)
PLA	73.7	49.3
Multiblock (PEG 200)	59.6	38.6
Multiblock (PEG 400)	19.2	2.0
Multiblock (PEG 1450)	17.6	0.6

In computer simulation **(Figure 8)**, the copolymer tends to show clear separation of the PLA and PEG domains. This spatial organization is confirmed by AFM Phase imaging microscopy of a copolymer film **(Figures 9 and 10)** showing a clear segregation between the PEG and PLA blocks.

Conclusion

PEG-ε-caprolactone form micelles easier than the triblock copolymers, hence the multiblock copolymer will possess enhanced efficiency for NS preparation. It is of growing interest to study the behavior of this new class of multiblock (-PLA-PEG-PLA-)$_n$ copolymer as a drug carrier for prolonged release of anti-infectious or anti-neoplasic drugs. Prior to be used as a new biomaterial, cytocompatibility and degradation studies must be conducted for safety.

Hence, the objectives of this study were to 1) conduct *in vitro* cytotoxicity tests on the new biomaterial; 2) manufacture NS from the (-PLA-PEG-PLA-)$_n$ multiblock copolymer; and 3) report the physico-chemical properties of the NS with regard to the size, zeta potential, porosity and hydrophilicity. Furthermore, incorporation of Rhodamine B as a drug model in the NS and its *in vitro* release were studied to assess the potential of these NS as a drug carrier.

Materials and Methods

<u>Materials</u>

Rhodamine B was purchased from Sigma (St Louis, MO, USA). Chloroform was obtained from Anachemia (Montreal, Qc, Canada). Poly (vinylalcohol) 80% hydrolyzed, sodium hydrogenophosphate 98%, sodium chloride 98%, and sodium azide were from Aldrich Chemical Company Inc., Minimum Essential Medium, Pyruvate substrate, Sigma color reagent, gentamycin, and MTT (dimethyl thiazoldiphenyltetrazoliumbromide) were from Sigma (St Louis, Mo, USA). Hanks' Balanced Salt Solution, fetal bovine serum, and trypsin-EDTA were obtained from Gibco Life Technologies (Burlington, Canada). Tetraphenyltin, adipic chloride, and pyridine were purchased from Aldrich (Oakville, ON, Canada).

<u>2) Polymer synthesis</u>

A triblock polymer was first synthesized by a ring-opening polycondensation of (DL)-Lactide in the presence of polyethylene glycol (PEG), as described by Cohn and Younes (*J. of Biomredical Materials Res.* **22**: 993-1009 (1988)). Briefly, 8.3 mmol of PEG (molecular weight 400) was added to 158.3 mmol of (DL)-Lactide (molecular weight 10000) in a round bottom single neck flask.

10. The stealthy polymeric biodegradable nanospheres according to claim 7 wherein said polyethylene is a polyethylene oxide (PEO).

11. The stealthy polymeric biodegradable nanospheres according to claim 10, wherein the polyethylene oxide (PEO) is a polyethylene glycol (PEG).

12. The stealthy polymeric biodegradable nanospheres according to any one of claims 7 to 11, wherein the dicarboxylic polyethylene is selected from the group of dichloride dicarboxylic (PEG) and dibromide dicarboxylic PEG.

13. The stealthy polymeric biodegradable nanospheres according to any one of claims 1 to 12, wherein the polyester (ii) is selected from the group consisting of polylactic acid (PLA), polylactic-co-glycolic (PLGA), polycaprolactone (PCL) and their copolymers.

14. The stealthy polymeric biodegradable nanospheres according to claim 13, wherein the polyester (ii) is polylactic acid (PLA).

15. The stealthy polymeric biodegradable nanospheres according to any one of claims 1 to 14, wherein the pharmaceutical compound (iii) is a drug, a protein and/or a nucleic acid molecule for the prevention or treatment of various diseases and/or delivery of different types of therapeutic agents.

16. The stealthy polymeric biodegradable nanospheres according to claim 15, wherein the therapeutic agents are selected from the group consisting of anticancer agents, immunosuppressive agents, agents for steroid therapy, anti-arrhythmic agents, antibiotics, antiparasitics, antivirals, antifungics, gene-therapy agents, antisense molecules, orphan drugs, and vitamins.

17. The stealthy polymeric biodegradable nanospheres according to any one of claims 1 to 16, wherein the nanosphere has an average size of less than 800 nm.

26. The polyester-polyethylene multiblock copolymer according to claim 25, wherein the polyethylene oxide (PEO) is a polyethylene glycol (PEG).

27. The polyester-polyethylene multiblock copolymer according to any one of claims 22 to 26, wherein the dicarboxylic polyethylene is selected from the group consisting of dichloride dicarboxylic (PEG) and dibromide dicarboxylic PEG.

28. A method for preparing the polyester-polyethylene multiblock polymer of formula (III) as defined in any one of claims 22 to 27, comprising the steps of:

a) oxidizing both terminal hydroxyl groups (-OH) of a polyethylene glycol into corresponding carboxylic groups (COOH) by means of a Jones reaction;

b) chlorinating the carboxylic functions of the polyethylene glycol obtained in step a) by making use of a $SOCl_2$ reagent so as to obtain a polyethylene glycol with terminal dichloride acid functions; and

c) reacting the polyethylene glycol having terminal dichloride acid functions obtained in step b) with the PLA-PEG-PLA triblock polymer obtained in claim 34 by making use of polycondensation reaction so as to obtain a multiblock copolymer as claimed in any one of claims 3 to 12.

29. An improved method for preparing a PLA-PEG-PLA multiblock copolymer of formula (I):

$$\text{ABA-(c-ABA)}_n\text{-c-ABA} \qquad \text{(I)}$$

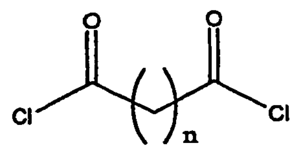
wherein

- n is a number equal or higher than 2;
- ABA is a PLA-PEG-PLA triblock; and
- c is a carboxylic diacid.

said method comprising the steps of:

a) preparing a PLA-PEG-PLA triblock;

b) mixing the PLA-PEG-PLA triblock prepared in step a) with a diacid of formula (II):



(II)

wherein n is a number equal to or greater than 1; and

c) subjecting the mixture of step b) to a polycondensation reaction with the presence of a dicyclohexylcarboxydiimide reagent and/or a chemical equivalent thereof, said catalyst improving the efficiency of the reaction, thereby allowing to obtain the requested multiblock copolymer.

30. The method according to claim 29, wherein step a) comprises the steps of:

(i) reacting at least one monomer A with at least one monomer B by a polycondensation reaction so as to produce a PLA-PEG-PLA triblock;

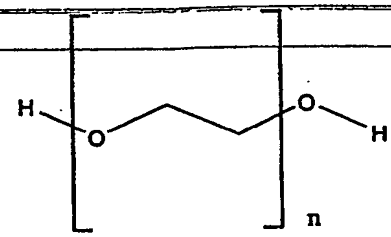
(ii) dissolving the PLA-PEG-PLA triblock obtained in step (i) in acetone;

(iii) precipitating the dissolved PLA-PEG-PLA triblock in step (ii) in water; and

(iv) washing and drying the PLA-PEG-PLA triblock polymer.

31. The method according to claim 30, wherein monomer A is selected from the group comprising of dioxanediones, lactones and dioxanones.

32. The method according to claim 30 or 31, wherein monomer B is a polyethylene glycol (PEG) represented by the formula (B):



wherein n represents a number between 200 and 2000.

33. The method according to any one of claims 30 to 32, wherein step (ii) is carried out with a tin based catalyst at a temperature between 160° C and 180° C under an inert atmosphere.

34. The method according to any one of claims 29 to 33, wherein the diacid chloride used in step b) is selected from the group comprising of propanedioic acid, butanedioic acid and pentanedioic acid.

35. The method according to any one of claims 29 to 34, wherein the chemical equivalent of dicyclohexylcarboxydiimide (DCC) is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC).

36. The method according to any one of claims 29 to 35, wherein the carboxylic diacid in step c) is selected, the group comprising of butanedioic acid, propanedioic acid and pentanedioic acid.

37. A method for delivering a pharmaceutical compound into a mammal, said method comprising the step of:

administering to the mammal a stealthy polymeric biodegradable nanosphere as claimed in any one of claims 1 to 20 loaded with a therapeutically effective amount of the pharmaceutical compound.

38. The method according to claim 37, wherein the pharmaceutical compound comprises a therapeutic agent which is selected from the group of anticancer agents, immunosuppressive agents, agents for steroid therapy, anti-arrhythmic agents, antibiotics, antiparasitics, antivirals, antifungics, gene-therapy agents, antisense molecules, orphan drugs, and vitamins.